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Holopelagic *Poeobius meseres* ("Poeobiidae," Annelida) Is Derived From Benthic Flabelligerid Worms

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Abstract. Phylogenetic relationships among and within the more than 70 recognized families of Annelida are poorly understood. In some cases, such as the monotypic Poeobiidae, derived morphology hinders the ability to find convincing synapomorphies that help elucidate evolutionary origins. In such cases, molecular data can be useful. Poeobiidae consists of the holopelagic polychaete *Poeobius meseres*, which is typically found in midwater depths off California. Morphologists have speculated that it is close to or within Flabelligeridae, but definitive evidence was lacking. Herein we use maximum likelihood phylogenetic reconstruction methods to examine the nuclear 18S rDNA (SSU) gene and the mitochondrial cytochrome *b* gene. Our results strongly support the hypothesis that *P. meseres* is a highly derived flabelligerid annelid closely related to *Therochaeta*. Thus, Poeobiidae is a junior synonym for Flabelligeridae. This result raises interesting questions about the evolution of the holopelagic *P. meseres* from a benthic ancestral flabelligerid.

Introduction

The holopelagic polychaete *Poeobius meseres*, which is up to 27 mm in length and has 11 poorly defined segments, is the sole recognized representative of Poeobiidae (Annelida; Fig. 1). It has been recorded from the eastern Pacific at depths of 350–1300 m (Rouse and Pleijel, 2001). The body is largely gelatinous with a thick mucus sheath, and segmentation is not clearly visible. The anterior end bears retractable pale green "tentacles," which consist of a pair of grooved palps and branchiae. Individuals of this species can be overlooked when taken because they are easily damaged by net tows and are amorphous out of water. However, some aspects of their biology are known (*e.g.*, metabolism—Thuesen and Childress, 1993; diet—Uttal and Buck, 1996). A second species, *Enigma terwillei*, has been attributed to Poeobiidae (Rouse and Pleijel, 2001), but it has only been collected once and the type material was lost. Therefore, it should be considered *incertae sedis* (of uncertain taxonomic position) as suggested by Fauchald (1977). The evolutionary origins of *P. meseres* are unclear, but there is speculation of a relationship to or within Flabelligeridae.

Flabelligeridae, known as bristle-cage worms, contains about 130 species in 14 recognized genera. These occupy many habitats ranging from intertidal zones to deep-sea muds, and are from 5 mm to 220 mm in length (Rouse and Pleijel, 2001). Members of Flabelligeridae are typically recognized by their unique and complex anterior end. The prostomium and peristomium are fused, forming a retractable head that in most cases (*e.g.*, *Flabelligera*, *Pherusa*, and *Diplocirrus*) is protected by a cage of modified setae from the first few parapodia (Spies, 1975; Blake, 2000). The body is often coated with sediment granules or a mucus sheath (Fauchald and Rouse, 1997).

Information on flabelligerid biology is limited. Spies (1977) found that *Flabelligera commensalis* retains gametes in the coelomic peritoneum until just before spawning. For comparison, many polychaetes shed their gametes directly into the coelom to mature. Studies of flabelligerid and *P. meseres* larvae are lacking (but see Spies, 1977). Some flabelligerids (*e.g.*, *Flabelligera affinis*) are motile surface-deposit feeders, whereas most others (*e.g.*, *Pherusa plumosa* and *Brada villosa*) are discretely motile, feeding in cracks and crevices (Fauchald and Jumars, 1979). Grooved palps and current-generating branchial fields are thought to be used in feeding (Fauchald and Jumars, 1979). Flabelligerids have a fossil record from about 295 million years ago in the Francis Creek Shale of Illinois (Carbondale Formation, Desmoinesian Series, Middle Pennsylvanian System of North America; Hay, 2002). Fossils have been placed into

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Figure 1. Individual of *Poeobius meseres* floating in the water column. Photo credit: MBARI ROV *Tiburon* and Karen Osborn.

one species, *Mazopherusa prinosi*, which is most similar to the extant *Pherusa* on the basis of the cephalic cage. One additional worm that deserves mention is *Flota flabelligera*, another pelagic polychaete. Hartman (1967) initially considered it a flabelligerid, but she later transferred it to Fauveliopsidae (1971). Recently, *Flota* has been considered to be closely related to taxa in Terebellida and especially to *Poeobius* (Rouse and Pleijel, 2001, 2003). Under the Linnean system, *Flota* is still considered a separate family. Neither *F. flabelligera* nor *P. meseres* is known to have a fossil record.

To our knowledge, a formal phylogenetic hypothesis has never been proposed for relationships within Flabelligeridae. The first flabelligerid (*Amphitrite plumosa*, now *Pherusa plumosa*) was formally described in 1776 by Müller, and the family was erected in 1894 by Saint-Joseph. The current taxonomic scheme for the group is based largely on the works of Haase (1915), Fauvel (1927), Støp-Bowitz (1948), Day (1961, 1967), and Hartman (1961, 1969). Recent morphological cladistic analyses (Rouse and Fauchald, 1997; Rouse and Pleijel, 2001) place Flabelligeridae within Cirratuliformia, Terebellida, and Canalipalpata. Relationships within this putative clade (also including Acrocirridae, Cirratulidae, Ctenodrilidae, Fauveliopsidae, Poeobiidae, and Sternaspidae) remain unclear.

With the recognition of Poeobiidae Heath 1930, the monophyly of Flabelligeridae has been called into question. There are morphological similarities between these two groups, such as the presence of a retractable head, a pair of grooved palps, an anterior ring of branchiae, and a gelatinous mucus sheath, although some of these features are not exclusive to these taxa. Fauchald and Jumars (1979) also thought that these two groups belong to the same feeding guild—filter-feeding, discretely motile, tentacular—because the palps of Poeobiidae resemble those of flabelligerids. *P. meseres* was originally thought by Heath (1930) to be the link between the Annelida and "Echiuroidea," but later Pickford (1947, cited in Robbins, 1965) labeled it an aberrant polychaete. Hartman (1955) first pointed out the remarkable resemblance *P. meseres* has to flabelligerids—for example, the cephalic structure and the thick gelatinous membrane around the trunk region. Dales (1962) considered *P. meseres* to be an offshoot of the flabelligerids, leading Robbins (1965) to suggest that the relationship between Flabelligeridae and *P. meseres* be assessed in detail due to morphological similarities. Rouse and Fauchald's (1997) morphological cladistic analysis consistently placed Flabelligeridae closer to Fauveliopsidae and Sternaspidae than to Poeobiidae. Later, Rouse and Pleijel (2003) suggested that the recognition of Poeobiidae renders Flabelligeridae paraphyletic by presenting a strict consensus tree in which *Poeobius* and *Flota* are nested among three flabelligerid taxa (*Brada*, *Pherusa*, and *Flabelligera*). They present an anterior branchial cluster as the only non-homoplastic morphological apomorphy to support the *Poeobius*/*Flota*/flabelligerid clade. Rouse and Pleijel acknowledge that "the exact nature of . . .[the extreme anterior branchial cluster] is still unresolved" (p. 181), and given that several other polychaete taxa have anterior branchiae/palps/tentacles, the nature of this apomorphy is tenuous. Furthermore, their analysis fails to assess the robustness of their recovered topologies over alternative hypotheses. Thus, the question of whether *P. meseres* falls within Flabelligeridae is still open to debate.

The purpose of this study is to examine the position of *P. meseres*. In doing so, we hope to begin examining relationships within Flabelligeridae. We examined data from two molecular markers, 18S rDNA and cytochrome *b*, using maximum likelihood. Our phylogenetic results will allow us to look for changes that accompany the transition from a benthic to a holopelagic mode of life.

Materials and Methods

Specimens

The taxa sampled in this study, their collection locality, voucher specimen number, and GenBank numbers are listed in Table 1. Limited taxon sampling in this study was due to the difficulty in obtaining samples from deeper waters. Three outgroups, *Sternaspis* sp., *Fauveliopsis glabra*, and *Cirratulus spectabilis* were chosen on the basis of recent studies (Rouse and Fauchald, 1997; Rouse and Pleijel, 2001). All specimens were stored at −80° C or at 4 °C in ethanol immediately after collection. For most specimens, species identification was made at the time of collection and subsequently confirmed by Sergio Salazar-Vallejo (Unidad

Table 1

Taxonomic inclusion, locality information and GenBank accession numbers

Taxon	GenBank Accession No. 18S	GenBank Accession No. Cyt *b*	Museum number	Collection site	Latitude	Longitude
Ingroup Taxa						
Brada villosa 1	AY708535		USNM 1073349	Central California, USA	36°50.860′N	121°53.749′W
Brada villosa 2*		AY727748	USNM 1073357	Trondheimsfjord, Norway	63°32.745′N	10°14.910′E
Diplocirrus glaucus 1	AY708533	AY727750	completely used	Geitnstranda, Trondheim, Norway	63°24.00′N	9°58.67′E
Diplocirrus glaucus 2*	AY708534	AY727751	USNM 1073353	West Gullmarsfjorden, Sweden	58°13.30′N	11°20.20′E
Flabelligera affinis 1	AY708531	AY727755	USNM 1073355	Central California, USA	36°49.847′N	122°01.729′W
Flabelligera affinis 2*	AY708532		USNM 1073354	Gullmarsfjorden, Sweden	58°13.154′N	11°24.416′E
Ilyphagus octobranchus	AY708530	AY727749	USNM 1073351	South of Woods Hole, Massachusetts, USA	39°54.134′N	70°35.054′W
Pherusa plumosa 1	AY708529	AY727756	USNM 1073348	South of Woods Hole, Massachusetts, USA	40°53.019′N	70°25.003′W
Pherusa plumosa 2	AY708528	AY727752	USNM 1073356	Central California, USA	36°49.847′N	122°01.729′W
Therochaeta collarifera	AY708527	AY727753	USNM 1073350	South of Woods Hole, Massachusetts, USA	39°54.134′N	70°35.054′W
Poeobius meseres	AY708526	AY727754	USNM 1073358	Monterey Canyon, California, USA	36°41.228′N	122°01.965′W
Outgroup Taxa						
Cirratulus spectabilis	AY708536	AY727746	USNM 1073359	Snug Harbor, Washington, USA	48°34.317′N	123°10.115′W
Fauveliopsis glabra	AY708537		USNM 1073352	South of Woods Hole, Massachusetts, USA	39°54.085′N	69°54.601′W
Sternaspis sp. '*Banyuls*'†	AB106264					

* Sample kindly provided by Fredik Pleijel.

† 18S rDNA *Sternaspis* data are from Hall *et al.* (2004).

Chetumal, Mexico). Fredrik Pleijel identified the three specimens that he kindly provided (Table 1). Herein we have decided to follow the taxonomy of Blake (2000). However, Salazar-Vallejo has noted that both *Brada villosa* and *Fabelligera affinis* may be over-synonymized. He mentioned that, given the older literature, the following designations are probably correct and may be re-erected in the near future: our *Brada villosa* 1 = *B. pluribranchiata* and our *Flabelligera affinis* 1 = *F. infundibularis*. Also *B. villosa* from Norway is probably referable to *B. pilosa*. Voucher specimens have been deposited in the Smithsonian Museum of Natural History (Washington DC).

Data collection

For most tissues, DNA was extracted using the DNeasy Tissue Extraction Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. However, for *Poeobius meseres*, a standard phenol/chloroform with proteinase K extraction procedure was used (Hillis *et al.*, 1996). The 18S rDNA gene and approximately 420 basepairs of the cytochrome *b* (cyt *b*) mitochondrial gene were amplified and sequenced using the primers listed in Table 2. Amplification *via* HotStart-PCR of 18S (thermocycler profile: 3 min 94 °C; addition of polymerase; 3 min 94 °C; 40 cycles: 1 min 94 °C, 1 min 30 s 40 °C, 2 min 30 s 72 °C; 1 cycle: 7 min 72 °C; reaction-mix: 10 m*M* Tris-HCl pH 9.0, 50 m*M* KCl, 0.1% Triton X-100, 2.5 m*M* $MgCl_2$, ~ 1 ng/μl genomic DNA, 0.4 m*M* dNTPs, 0.8 μM of each primer, 0.03 U/μl *Taq* DNA Polymerase (Promega, Madison, WI)) was carried out in a volume of 25 μl. The cyt *b* protocol used the same recipe but a different thermocycling protocol (3 min 94 °C; addition of polymerase; 2 min 94 °C; 15 cycles: 30 s 94 °C, 30 s 42 °C, 30 s 72 °C; 20 cycles: 30 s 94 °C, 30 s 37 °C, 45 s 72 °C; 1 cycle: 7 min 72 °C). PCR products were isolated on 1% agarose gels. The appropriate-sized bands were excised and purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Purified products were quantified using a mass standard on 1% agarose gels. Bi- directional Sequences were determined on an ABI Prism 3100 (using the BigDye ver. 3.0 kit, Applied Biosystems, Foster City, CA) or a Beckman CEQ 8000 (using one-half reactions of the CEQ DTCS Quick Start Kit, Beckman Coulter, Fullerton, CA).

Sequences were assembled and edited using the LaserGene package (DNAStar, Inc., Madison, WI). The sequences were then aligned using ClustalX (Thompson *et al.*, 1997). The alignment was corrected by eye in MacClade ver. 4.06 (Maddison and Maddison, 2003). Regions of the 18S data that could not be unambiguously aligned were excluded from the analyses. The cyt *b* alignment was translated to inferred amino acid sequence using the *Drosophila* mitochondrial DNA code to check for sequencing errors. Alignments (Accession #S1268 and #M2212–2214) are available at TREEBASE (www.treebase.org).

Phylogenetic analysis

Phylogenetic analyses used maximum likelihood (ML) methods for both data sets individually and for combined

Table 2

Nucleotide primers used in amplification and sequencing

Primer	Sequence 5′-3′	Reference
18S rDNA		
Amp. & Seq.		
18e	CTG GTT GAT CCT GCC AGT	Hillis and Dixon, 1991
18R	GTC CCC TTC CGT CAA TTY CTT TAA G	Halanych, unpublished
18O	GGA ATR ATG GAA TAG GAC C	Halanych *et al.*, 1998
18R1779	TGT TAC GAC TTT TAC TTC CTC TA	Struck *et al.*, 2002
18P	TAA TGA TCC TTC CGC AGG TTC ACC T	Hillis and Dixon, 1991
Sequencing only		
18L	GAA TTA CCG CGG CTG CTG GCA CC	Hillis and Dixon, 1991
18H	AGG GTT CGA TTC CGG AGA GGG AGC	Hillis and Dixon, 1991
18F509	CCC CGT AAT TGG AAT GAG TAC A	This study
18F997	TTC GAA GAC GAT CAG ATA CCG	This study
18R925D	GAT CYA AGA ATT TCA CCT CT	This study
18R993D	CTT GRC AAA TGC TTT CGC	This study
18Q	TGT CTG GTT AAT TCC GAT AAC	Halanych *et al.*, 1998
18Qϕ	GTT ATC GGA ATT AAC CAG ACA	Halanych *et al.*, 1998
18F1435	AGG TCT GTG ATG CCC TTA GAT	This study
Cytochrome b		
Amp. & Seq.		
Cytb 424F (RT-1)	GGW TAY GTW YTW CCW TGR GGW CAR AT	Boore and Brown, 2000
Cytb 876R (RT-2)	GCR TAW GCR AAW ARR AAR TAY CAY TCW GG	Boore and Brown, 2000
Sequencing only		
cobr825	AAR TAY CAY TCI GGY TTR ATR TG	Jennings and Halanych, 2005

data. For cyt *b*, nucleotide data with and without third positions were analyzed. Combined analyses used cyt *b* without third positions. ML analyses with a heuristic search of 100 random sequence addition replicates and tree-bisection-reconnection (TBR) were conducted using PAUP* 4.0b10 (Swofford, 2002). ModelTest 3.06 (Posada and Crandall, 1998) was used to select the model of evolution with the best fit for each data set (see below). Nodal support was estimated using a bootstrap analysis consisting of 1000 heuristic iterations with random sequence addition.

In addition, using MrBayes (Huelsenbeck and Ronquist, 2001), we conducted a Bayesian inference analysis on the inferred amino acid data to determine if there was additional phylogenetic information in the cyt *b* data. To do this, we inferred amino acid residues using the *Drosophila* mtDNA translation code. The Bayesian search employed the amino acid model with all the priors set to default settings. Two million generations were run with four chains (3 cold and 1 hot) that were sampled every 200 generations.

The constraints option within an ML heuristic search (as above) was used to generate topologies consistent with alternative hypotheses. Resultant trees were compared with unconstrained ML results using the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) implemented in PAUP* 4.0b10. Only the 18S data set was used because multiple outgroups allowed for testing the monophyly of Flabelligeridae, including *P. meseres*.

Results

The 18S rDNA data set included 13 terminal taxa and 2106 characters. Of the 1474 nucleotide positions that could be unambiguously aligned, 28.1% (414 positions) were variable and 18.3% (270 positions) were parsimony informative. ModelTest indicated a TrNef+Γ model (nucleotide frequencies equal; rates A⇔C 1.0000, A⇔G 2.2477, A⇔T 1.0000, C⇔G 1.0000, C⇔T 3.6285, G⇔T 1.000; gamma shape parameter = 0.2552) for the ML analysis. The single best ML tree (Ln likelihood = −6283.79641) is shown in Figure 2. A monophyletic Flabelligeridae clade including *Poeobius meseres* was recovered with a bootstrap value of 100%. Specifically, *P. meseres* falls as a sister taxon to *Therochaeta collarifera* (bootstrap 100%). The result that *Ilyphagus octobranchus* appears to be very closely related to *Diplocirrus glaucus* (bootstrap = 98%) is noteworthy.

The cytochrome *b* nucleotide data set consisted of 11 terminal taxa and 404 characters; the number of characters was reduced to 318 once the ends were trimmed. Of those, 57.2% (182 positions) were variable and 42.1% (134 positions) were parsimony informative. ModelTest indicated a TVM + Γ + I model (nucleotide frequencies A = 0.28290, C = 0.24680, G = 0.09230, T = 0.37800; rates A⇔C 3.3592, A⇔G 34.9373, A⇔T 15.4450, C⇔G 8.4295, C⇔T 34.9373, G⇔T 1.000; proportion of invariant sites = 0.3275; gamma shape parameter = 0.9565) for this analysis.

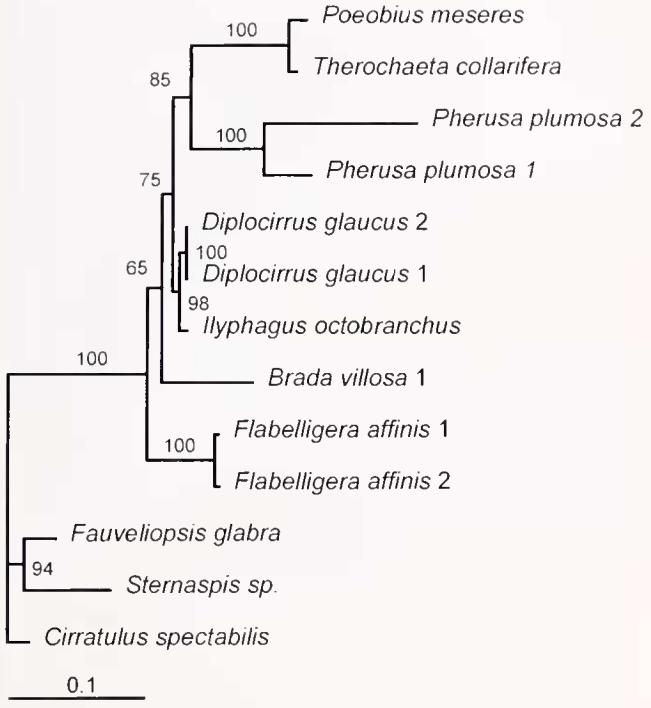
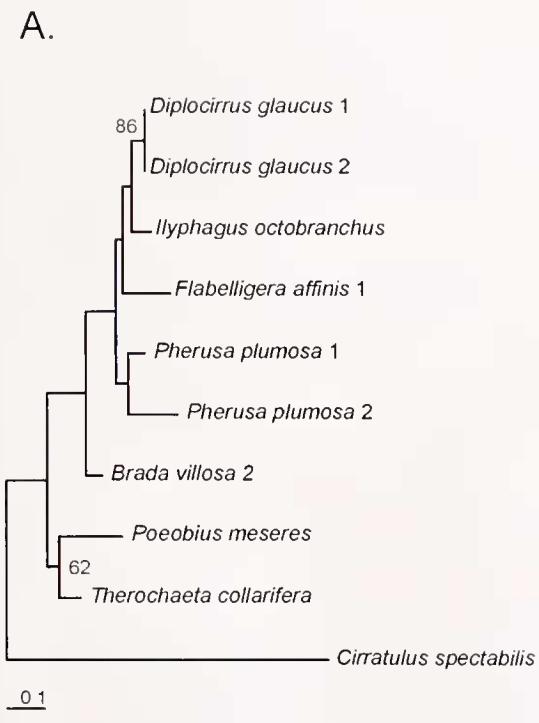


Figure 2. The single best 18S maximum likelihood tree (Ln likelihood = −6283.79641) based on 18SrDNA data set (see text for details). Bootstrap support values (>50%) out of 1000 iterations are represented at each node.

When third positions were included, a single best ML tree (Ln likelihood = −2157.13859) placed *P. meseres* within Flabelligeridae, but there was only weak (50%) bootstrap support (not shown). However, third positions appeared saturated because 94.3% (100 of 106 positions) of them displayed variation. Therefore, an ML analysis minus the third position was also performed, using a K81uf + Γ model with nucleotide frequencies A = 0.24400, C = 0.22550, G = 0.15250, T = 0.37800, rates A⇔C 1.0000, A⇔G 5.2849e+06, A⇔T 2.0229e+06, C⇔G 2.0229e+06, C⇔T 5.2849e+06, G⇔T 1.000, and gamma shape parameter = 0.2079. This tree (Ln likelihood = −962.14976; Fig. 3A) also grouped *P. meseres* with *Therochaeta collarifera*, but with a low bootstrap value (62%). As in the 18S analysis, *Ilyphagus octobranchus* and *Diplocirrus glaucus* are sister taxa, but with a bootstrap support less than 50%. Similarly, the Bayesian analysis of the amino acid data was poorly supported. Thus, for the sake of simplicity, that analysis is not described herein.

For the cyt *b* dataset, the percent divergence of the European and North American *Pherusa plumosa* is notable (10.8% for uncorrected distance). Further analysis is needed to assess whether the current nomen "*Pherusa plumosa*" represents multiple species.

The combined dataset consisted of 10 taxa with 2424



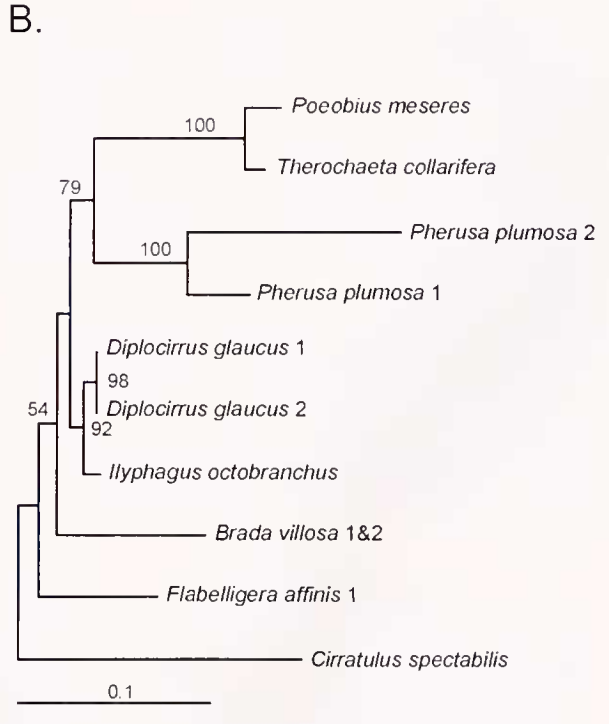


Figure 3. (A) The single best maximum likelihood tree (Ln likelihood = −962.14976) based on the cyt *b* nucleotide data set minus third positions (see text for details). (B) The single best maximum likelihood tree (Ln likelihood = −5810.60410) based on a combined data set of 18SrDNA and cyt *b* minus third positions (see text for details). For both trees, bootstrap support values (>50%) out of 1000 iterations are represented at each node.

Table 3

Shimodaira-Hasegawa results comparing alternative hypotheses[1]

Alternative hypotheses tested	Ln likelihood score	*P* value
Best Tree	−5369.97048	
Poeobius meseres outside Flabelligeridae	−5754.28014	<0.001*
Poeobius meseres & *Therochaeta collarifera* not sisters	−5466.10841	<0.001*
Ilyphagus octobranchus & *Diplocirrus glaucus* not sisters	−5376.96342	0.666

[1] Alternative hypotheses are written contrary to what is observed in best tree. Thus *P* values represent significance of support for relationships observed in the "best tree" over these alternatives.

* $P < 0.05$.

nucleotide characters. When ambiguously aligned positions and cyt *b* third positions were excluded (1687 nucleotides remaining), 28.1% (474 positions) were variable and 14.5% (245 positions) were parsimony informative. Because of data availability, only one outgroup (*Cirratulus spectabilis*) was used, accounting for the lower number of variable sites when compared to the 18S dataset. The model for the combined data was a GTR + Γ + I using the following parameters: nucleotide frequencies A = 0.26400, C = 0.21870, G = 0.25330, T = 0.26400; rates A⇔C 1.2605, A⇔G 2.4146, A⇔T 3.2160, C⇔G 0.7243, C⇔T 7.5504, G⇔T 1.000; proportion of invariant sites = 0.393; gamma shape parameter = 0.5704. Given the absence of *Fauveliopsis glabra, Sternaspis* sp., and *Flabelligera affinis,* the topology of the combined data tree (Ln likelihood = −5810.60410; Fig. 3B) was identical to the 18S results, with a monophyletic Flabelligeridae including *P. meseres* as sister to *Therochaeta collarifera.* There was also strong support for *Ilyphagus octobranchus* with *Diplocirrus glaucus* (bootstrap = 92%).

Table 3 presents Shimodaira-Hasegawa results of the hypotheses tested. The alternative hypotheses that *P. meseres* is neither a flabelligerid ingroup taxon nor the sister taxon of *Therochaeta collarifera* are significantly worse than the best hypothesis. However, the hypothesis that *Ilyphagus octobranchus* and *Diplocirrus glaucas* are not sister taxa to each other is not significantly worse than the best tree.

Discussion

All analyses described herein are consistent with placement of the holopelagic *Poeobius meseres* within Flabelligeridae, and specifically as sister taxon to *Therochaeta collarifera.* Even though only about half (6) of the recognized flabelligerid genera are included in our study, support that *P. meseres* is within the Flabelligeridae is very strong on the basis of bootstrap proportions (100%) and a Shimodaira-Hasegawa test ($P < 0.001$). Morphological studies suggest that *P. meseres* falls within or close to the flabelligerids (Hartman, 1955; Robbins, 1965; Rouse and Fauchald, 1997; Rouse and Pleijel, 2001, 2003; S. Salazar-Vallejo, Unidad Chetumal, Mexico, pers. comm.). Because of the agreement in morphological and molecular data, we formally propose that *Poeobius meseres* be referred to Flabelligeridae Saint-Joseph, 1894. This action makes "Poeobiidae" (Heath, 1930) a junior synonym of Flabelligeridae Saint-Joseph, 1894.

Two additional holopelagic worms, *Enigma terwillei* and *Flota flabelligera,* deserve mention in making this placement of *P. meseres.* First, as stated earlier, *E. terwillei* should be considered *incertae sedis.* However, if the initial and only description was accurate, it should also be referred to Flabelligeridae in the spirit of Rouse and Pleijel (2003). Second, although the suggestion that *Flota* is closely related to *Poeobius* (Rouse and Pleijel, 2003) awaits confirmation by definitive synapomorphies or a well-supported phylogenetic hypothesis, if it is correct, we must consider the possibility that there has been a radiation of holopelagic forms within Flabelligeridae.

The placement of *P. meseres* within Flabelligeridae is evolutionarily interesting because it suggests that this holopelagic species evolved from a benthic form. A notable change that presumably accompanied this transition was a reduction in thickness of the body wall that allowed *P. meseres* to be largely transparent. Many animals have transparent planktonic forms in which subcellular structures are arranged to reduce their reflectivity and scattering of light (Johnsen, 2001). For example, many transparent organisms cover the external surface with microscopic protrusions to reduce reflectivity. "Extracellular tissues" also help to lower scattering. On the basis of Johnsen's work, we predict that similar subcellular rearrangement will be observed in *P. meseres.*

The evolutionary origins of *P. meseres* may be the result of heterochrony during development. Although little is known about larval development in most flabelligerids, Spies (1977) investigated *Flabelligera commensalis* and concluded that there was evidence for midwater spawning. During the 11-setiger stage of *F. commensalis,* bristles form on the anterior end, and development of adult setae progresses from the anterior to the posterior. Additionally, *F. commensalis* has feeding larvae (Spies, 1977). How much similarity exists between the adult form of *P. meseres* and, for example, a larval stage of *Therochaeta* is not clear, and more complete larval and developmental studies need to be completed. Nonetheless, what is known is suggestive of heterochrony, specifically progenesis. *P. meseres,* in the transition from a benthic to a pelagic form, has lost all obvious external segmentation, including setae. Hartman (1955) observed that the only evidence of segmentation is the midventral ganglia with 11 apparent segments (see also

Heath, 1930). If the development of *F. commensalis* can be generalized to other flabelligerids, then the ancestor of *Poeobius* may have been a benthic flabelligerid with midwater spawning and relatively late development of setae (*i.e.*, at or after the 11-setiger stage). Given a feeding larvae as seen in *F. commensalis*, then selective forces favoring a prolonged planktonic stage (*e.g.*, lower risk of predation than metamorphosed juveniles, more consistent food supply, better opportunity to encounter mates) could have easily favored the evolution of a *Poeobius*-like organism.

Among annelids, there are presumably other examples of transition from benthic to pelagic form; these include *Flota*, alciopids, lopadorhynchids, isopilids, *Pontodora*, typhloscolecids, and tomopterids. All of these forms have retained segments with well-developed parapodia. Most of them are presumed to be active swimmers in the water column. These attributes are characteristic of Phyllodocida, the major annelid clade in which these taxa are placed (except *Flota;* Rouse and Pleijel, 2001). Because we do not understand the exact phylogenetic placement of these groups, it is hard to hypothesize the mechanism by which they evolved a holopelagic lifestyle. Although progenesis cannot be ruled out, some of these organisms may have evolved directly from an adult form (*e.g.*, tomopterids). In the case of *P. meseres*, the lack of adult structures argues for a larval origin.

Even though cyt *b* data are largely unresolved, 18S provides information that allows us to begin to address flabelligerid systematics. *Ilyphagus* groups with *Diplocirrus*, a result consistent with observed morphology (Hartman, 1965; S. Salazar-Vallejo, pers. comm.). Furthermore, these taxa displayed the lowest cyt *b* intergeneric divergence values (7.08%, range of others 9.0%–19.3%) within Flabelligeridae. In the Shimodaira- Hasegawa tests, the best tree that did not have *Ilyphagus octobranchus* and *Diplocirrus glaucus* as sister taxa made them a polytomy rather than splitting them apart. This may explain the disparity between high bootstrap support and a nonsignificant Shimodaira-Hasegawa value. To better elucidate the relationships within Flabelligeridae, we are currently working on obtaining a wider representation of taxa for molecular analyses.

We had hoped that the combination of a more conserved gene (18S) and a more quickly evolving gene (cyt *b*) would provide phylogenetic information at several levels. Unfortunately, cyt *b* seems to provide little information at either the nucleotide or amino acid levels. For the question at hand, synonymous positions have apparently experienced saturation by multiple mutations, thereby obliterating the phylogenetic signal, and the nonsynonymous positions have not changed enough to record shared history. This situation has been reported previously in cyt *b* (Halanych and Robinson, 1999). The only other polychaete paper that we know to have employed cyt *b* for evolutionary history is Breton *et al.* (2003), but those authors used mainly third positions to infer history within a species in a phylogeographic context. Cyt *b* was not useful in our study, but it has potential for being a useful phylogentic marker, and we would encourage others to explore the gene in other annelid groups.

In summary, our results provide strong support for placement of *P. meseres* within a monophyletic Flabelligeridae. Thus, "Poeobiidae" (Heath, 1930) is an invalid name because it is a junior synonym to Flabelligeridae Saint-Joseph, 1894.

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